INHALATION TOXICITY OF SARIN (GB) VAPOR IN THE GOTTINGEN MINIPIG: LOW-LEVEL THRESHOLD EFFECTS

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ABSTRACT

The goal of these experiments was to determine the lowest sarin (GB) vapor concentration of physiological significance. Gottingen-minipigs were individually exposed to concentrations of GB vapor ranging from 0.03 – 2.0 mg/m^3 for 60 minutes. Pupil constriction was assessed, under dim-light conditions, using an infrared light sensitive video camera and capturing high-resolution images of the eye before, during and after exposure. Pupil area was then quantified automatically off-line using a custom-designed software package. Pupil area was graphed as a function of time. The relationship between the dose-response curve (percent pupil constriction as a function of time) and vapor concentration was investigated.

INTRODUCTION

In order to estimate the potential impact of a chemical warfare agent vapor contamination on battlefield operations, it is necessary to fill critical gaps in data involving acute low-level exposures. While the initial signs of such an exposure may not be immediately life threatening, it is possible that they may become a physiological dysfunction, affecting performance of some military tasks. The ability to estimate threshold conditions of exposure (concentration and duration) that are likely to result in physiologically significant effects is an essential prerequisite for predicting the potential impact on task performance or military operations. The primary focus of these studies is to fill gaps in toxicological databases necessary for designing materiel for the chemical battlefield. Traditionally, the military and other organizations dealing with inhalation toxicology have accepted the principle of dosage (concentration(C) x time (t)) as constant over time when assessing the impact of nerve agent vapor exposures (Haber's law, 1924). Thus, Haber's law was used to extrapolate the bulk of dose-response data (based upon relatively short exposure times) to predict response probabilities involving longer exposure times. However, this concept has been found to be inadequate for assessing biological effects from exposure to many acutely toxic gases and aerosols (ten Berge et al., 1986). The Reutter and Wade report (1994) brought to light the inadequacies of the current research database necessary for defending the soldiers of today. Recent efforts (Mioduszewski et al., 2002, among others) have resulted in data which includes low concentration exposures over long times which can best be described with a toxic-load model (ten Berge et al., 1986). Continued research is needed to generate data to fill the data gaps in the

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low-level inhalation nerve agent toxicology database. The military and scientific community have been at odds over what constitutes a "low-level" nerve agent exposure and filling holes in the existing nerve agent toxicology database is prerequisite to answering related questions vital for troop protection: What are the limits of nerve agent exposure that need to be detected for fear of those personnel exposed suffering degradation in job performance? When is it "safe" to come out of protective posturing? How dirty is clean enough? How long can a low-level exposure be tolerated before the signs become life threatening? Therefore, it is imperative to experimentally define nerve agent exposure levels that can be considered the threshold between "no observable effect" and "measurable biological effects". During a whole-body exposure to GB vapor, the first noticeable effect, at such concentrations, is constriction of the pupil (miosis).

In order to develop models for predicting the probability of toxicity of low-level nerve agent exposures for different concentrations and durations of exposure, additional data from a non-rodent species is needed. The pig was chosen as our model for studying the effects of GB vapor on the pupil because of anatomical and physiological similarities to humans (see Information Resources for Swine in Biomedical Research (USDA, 2000) for a comprehensive review). The dimensions of the existing 1000-L whole-body exposure chamber required the use of a miniature swine (Gottingen strain) because of the small size of sexually mature young adults. During whole body GB exposures the minipigs were secured in a sling apparatus that allowed continuous monitoring of at least one eye. The pigs were individually exposed to fixed concentrations of GB vapor ranging from 0.03 – 2.0 mg/m^3 for 60 minutes. Pupil constriction was assessed, under dim-light conditions, using an infrared light sensitive video camera and image acquisition hardware/software. Pupil size was continuously monitored by capturing high-resolution images of the eye before, during and after exposure to GB vapor. Pupil area was then quantified automatically off-line using a custom-designed software package. Pupil area was graphed as a function of time and the EC_{50} (miosis) was calculated.

METHODS

ANIMALS

Male 4-6 month old (9-12 kg) Ellegaard Gottingen minipigs (Marshall Farms, NY) were used in these studies. A silicone catheter (Bard access systems, 6.6 Fr.) was implanted in the right external jugular vein. During nerve agent exposures, the catheter was maintained by a continuous i.v. infusion of lactated Ringers solution and blood samples were sequentially withdrawn periodically.

SLING RESTRAINT

The frame of the sling was constructed of airtight stainless steel pipe and Swagelok™ fittings. The slings were custom designed (Lomir Biomedical, Inc., Malone, NY) to fit the frame and the size of the pigs used in the studies. The sling was constructed of canvas and fitted to accept the animal through 4 leg holes. The pig was maintained in the sling by 2 straps that secured over the pig's shoulders and hips. A muzzle harness was placed over the animal's snout, and secured both laterally and ventrally to the stainless-steel framing, and prevented the animals from moving their heads from side-to-side. This enabled us to maintain a consistent angle and distance from the infrared (IR) camera to the animal's eye. The harness was fitted so that it did not interfere with the animal's ability to open its' mouth to breath.

CHEMICALS

Isopropyl methyl phosphonofluoridate (Sarin or GB) was used for all vapor exposures in this study. Chemical agent standard analytical reagent material (CASARM)-grade GB was verified (usually 98.3 + 0.48 wt. % pure as determined by quantitative ^{31}P-NMR) and stored in sealed ampoules containing

nitrogen. Ampoules were opened as needed to prepare external standards or to be used as neat agent for vapor generation. All external standards for GB vapor quantification were prepared on a daily basis. Triethylphosphate (99.9% purity), obtained from Aldrich Chemicals, Milwaukee, WI, was used as the internal standard for the GB purity assays.

VAPOR GENERATION

Saturated GB vapor streams were generated by flowing nitrogen carrier gas through a glass vessel (multi-pass saturator cell) that contained liquid GB. The saturator cell consists of a 100-mm long, 25-mm o.d. cylindrical glass tube with two (inlet, outlet) vertical 7-mm o.d. tubes connected at each end. The main body of the saturator cell contains a hollow ceramic cylinder that serves to increase the contact area between the liquid nerve agent and the nitrogen. The saturator cell allows nitrogen to make three passes along the surface of the wetted ceramic cylinder before exiting the outlet arm of the glass cell. The saturator cell body was immersed in a constant temperature bath so that a combination of nitrogen flow and temperature could regulate the amount of nerve agent vapor going into the inhalation chamber. The entire apparatus was contained within a generator box that was mounted at the top of the inhalation chamber. Typically, the saturator cell was loaded with 2-4 ml of liquid nerve agent (CASARM grade). To maintain the integrity of the liquid nerve agent within the cell, a continuous low flow rate (1-2 ml/min) nitrogen stream was used. This setup was capable of precisely generating GB vapor over a concentration range of 0.001-2.0 mg/m^3.

INHALATION CHAMBER

Whole body exposures were conducted in a 1000-liter dynamic airflow inhalation chamber. The Rochester style chamber is constructed of stainless steel with Glass or Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.50" H_2O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor chamber airflow at the chamber outlet.
Two sampling methods were used to monitor and analyze the GB vapor concentration in the exposure chamber. The first method was a quantitative technique using solid sorbent tubes (Tenax/Haysep) to trap GB, followed by thermal desorption and gas chromatographic (GC) analysis (HP Model 6890, Agilent Technology, Baltimore, MD). The second method was a continuous monitoring technique using a phosphorus monitor (HYFED, Model PA260 or PH262, Columbia Scientific, Austin, Texas). Output from the HYFED provided a continuous strip chart record of the rise, equilibrium, and decay of the chamber vapor concentration during an exposure.

All samples were drawn from the same area (middle) of the chamber. Solid sorbent tube samples were drawn after the chamber attained equilibrium (t_{99}), while the HYFED monitored during the entire run. Solid sorbent tube samples were drawn from the chamber approximately every 10 min with each sample draw lasting 1-5 min depending upon chamber concentration and duration of exposure.

INFRARED CAMERA AND DATA COLLECTION

A Sony CCD black and white video camera (model XC-ST50) equipped with (2) IR 100-candlepower spotlights was focused on the animal's left pupil for the duration of the nerve agent exposure. Sequential images of the eye, under very low-level light conditions, were digitally captured for analysis and calculation of pupil area at a later time. All GB exposures were for 60 minutes. However, the pigs were required to remain in the exposure chambers for an additional 15 minutes for out-gassing. The pigs were then removed from the chamber and images were captured for an additional 30 minutes to ensure there was no further decrease in pupil area.

RESULTS

The captured images reveal a bright pupil surrounded by a darker iris. A program designed in LabView was utilized to calculate the area of the bright pupil based on the differences between screen coordinates using the equation for the area of an ellipse; area= A*B*π, where A is the horizontal radius and B is the vertical radius

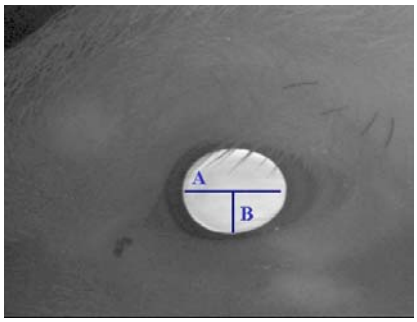

Figure 1. This figure shows a computer captured infrared image of a pig's pupil. The area of the pig's pupil is calculated based on the horizontal and vertical radiuses.

A minimum of 5 digital images of each pig's pupil, were captured under low-light conditions prior to beginning exposure to GB. Images were then captured repeatedly for the extent of the exposure (see figure 2 for a progression of images throughout the exposure).

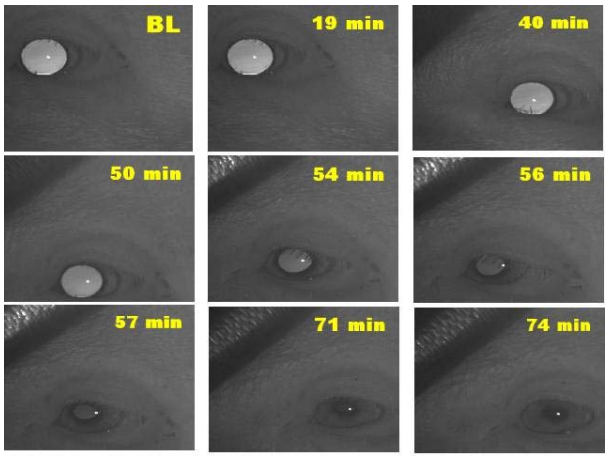

Figure 2. This figure shows the images of the progression of pupil constriction during a 60-minute exposure to GB at a concentration of 0.047 mg/m^3.

The baseline and subsequent images were quantified, as described above, and the pupil areas were calculated, off-line, and graphed vs. time from the onset of GB exposure in each individual pig (see figure 3 for such an example). The definition of miosis used in these studies was a decrease in pupil area to at or below 50% of the baseline mean.

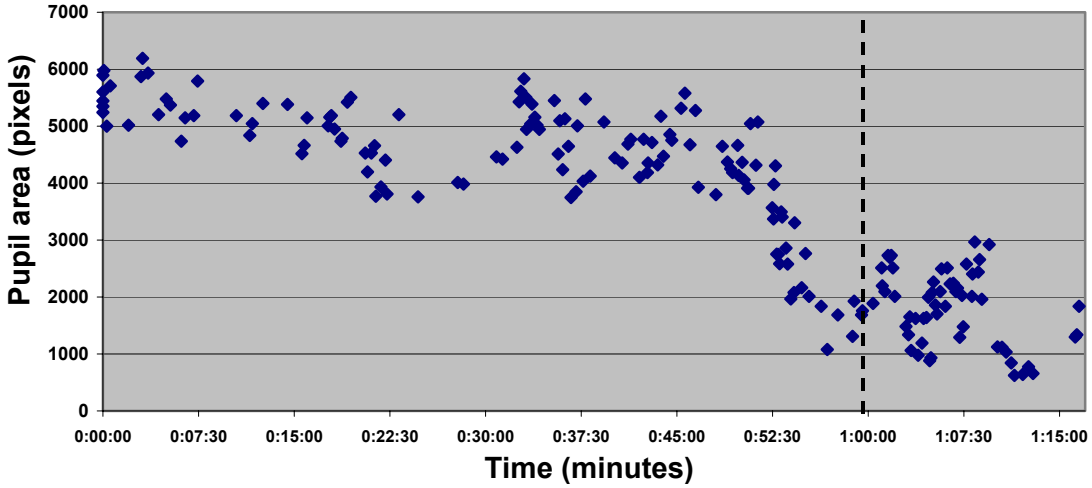

Figure 3. Pupil area vs. time of GB exposure. This figure is a typical graph of pupil area vs. time of GB exposure. This particular animal received a 60-minute exposure to 0.047 mg/m^3 GB. The end of the exposure time is indicated on the figure by the dashed vertical line. Each point represents the pupil area calculated from 1 captured image. The baseline mean was calculated from all of the images captured before beginning GB exposure. Pupil area was decreased to 50% of baseline area in this animal at approximately 57 minutes from the beginning of the experiment.

The results of 7 separate animal exposures to different concentrations of GB are shown in Table 1. Table 1 shows the summary of exposure concentrations and whether or not they caused 50% decrease in pupil area. For those concentrations that elicited a 50% reduction in pupil area the estimated time to 50% miosis is shown.

Table 1. Summary Of 60-Minute Gb Exposures

Pig #	**Conc. (mg/m^3)**	**Ct (mg/min/m^3)**	**50 % miosis?**	**Time to 50% miosis (min.)**
2	0.030	1.80	No	-
7	0.037	2.2	No	-
8	0.041	2.46	No	-
10	0.042	2.52	No	-
4	0.047	2.82	Yes	57
9	0.047	2.82	Yes	52
3	0.060	3.60	Yes	45

The definition of miosis used in these studies was a decrease in pupil area to at or below 50% of the baseline mean. The 3 exposures conducted with concentrations above 0.047 mg/m^3 resulted in miosis. The data included in the table were used to calculate an estimated EC_{50} (miosis) for a 60-minute GB exposure in the Gottingen minipigs using the acute oral toxicity up- and-down procedure (guideline 425)

statistical program (version 1.0). The calculated EC_{50} (miosis) was 0.04267 mg/m^3 with 95% confidence intervals of 0.041 to 0.047 mg/m^3.

CONCLUSIONS

The goals of the present experiments were two-fold: 1) Evaluate the use of IR technology for quantifying miosis under low-light conditions and 2) to use this technology to generate data to be used for filling in critical data gaps involving acute low-level nerve agent exposures. The IR camera allowed us to make sequential, real time assessment of the pupils under low-light conditions. Additionally, the insertion of a jugular catheter in the pigs prior to GB exposure allows us to take concomitant real-time blood samples that can be used to look for correlations between miosis and internal GB concentrations (Jakubowski, et al., 2001). The technique and animal model described within this paper can serve as a valuable tool for predicting the probability of toxicity of low-level nerve agent exposures for different concentrations and durations of exposure.

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